H. Wormald

G-526

THE QUESTION OF NUCLEAR FUSIONS IN THE BLACKBERRY RUST CAEOMA NITENS

BY

B. O. DODGE AND L. O. GAISER

(Contribution from Bureau of Plant Industry)

Reprinted from JOURNAL OF AGRICULTURAL RESEARCH

Vol. XXXII, No. 11 : : : Washington, D. C., June 1, 1926

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF LAND-GRANT COLLEGES

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1926

JOINT COMMITTEE ON POLICY AND MANUSCRIPTS

FOR THE UNITED STATES DEPARTMENT OF AGRICULTURE

R. W. ALLEN, CHAIRMAN
Chief, Office of Experiment Stations

C. L. MARLATT
Chairman, Federal Horticultural Board, and Associate Chief, Bureau of Entomology

C. L. SHEAR
Senior Pathologist in Charge, Plant Disease Survey and Pathological Collections

FOR THE ASSOCIATION OF LAND-GRANT COLLEGES

R. W. THATCHER
Director, New York Experiment Stations

H. W. MUMFORD
Dean, Illinois College of Agriculture, and Director of Experiment Station

S. B. HASKELL
Director, Massachusetts Experiment Station

EDITORIAL SUPERVISION

M. C. MERRILL
Editorial Chief of Publications, United States Department of Agriculture

All correspondence regarding articles from State experiment stations should be addressed to R. W. Thatcher, Agricultural Experiment Station, Ithaca, N. Y.

Published on the first and fifteenth of each month. This volume will consist of twelve numbers and the Contents and Index.

Subscription price: Domestic, $4.00 a year (two volumes)
Single numbers, 20 cents
Foreign, $5.25 a year (two volumes)
Single numbers, 25 cents

If separates are desired in quantity, they should be ordered at the time the manuscript is sent to the printer; single copies may be purchased until the supply is exhausted; in either case they will be supplied at cost.

Address all correspondence regarding subscriptions and purchase of numbers and separates to the Superintendent of Documents, Government Printing Office, Washington, D. C.

THE QUESTION OF NUCLEAR FUSIONS IN THE BLACKBERRY RUST, CAEOMA NITENS[1]

By B. O. Dodge, *Pathologist*, and L. O. Gaiser, *Junior Pathologist, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Strains of such obligate parasites as the mildews, smuts, and rusts, which are particularly pathogenic only when sowed on certain varieties of a host species, are now recognized as specialized or biologic forms. If the same condition obtains in case of the blackberry orange rust the conflicting reports coming in from observers in different regions would in part be understood. The writers have in culture several strains of this Rubus rust which can be readily distinguished. Moreover, the rust as it exists in certain plants is of such a nature as to suggest either that some strains are unstable or that the mycelia of two distinct strains may intermingle in the same sorus primordium (*5*).[2] Should the former be true, one is forced to explain how such an unstable strain could arise. If the latter be true, then a situation more conducive to the breeding of a hybrid rust as suggested in the paper referred to would be difficult to imagine. What the result of such a hybrid union would be depends first of all upon what becomes of the two nuclei which are brought together in a spore in consequence of the cell fusions. Should the nuclei fuse, as has been claimed, reduction would follow, and one might look for the effects of the crossing in the next generation. If, however, no nuclear fusion takes place, what then is the nature of the four sporidia? In prosecuting further studies, therefore, on the susceptibility of blackberry varieties to races of the orange rust, it has been found desirable to know exactly what the nuclei do during spore formation and germination. The life histories of the Endophyllum type of rusts and that of the short-cycled or *Caeoma nitens* rusts of blackberry are very similar. A knowledge of nuclear behavior in one group should lead to a clearer understanding of what takes place in the other group. A more detailed cytological investigation of the aeciospores of the Rubus rust during their development and germination has been made recently. The results of this study are reported at this time.

[1] Received for publication Oct. 14, 1925; issued June, 1926.
[2] Reference is made by number (italic) to "Literature cited," p. 1024.

REVIEW OF LITERATURE

The Moreaus, in a series of papers, the last of which appeared in 1919 (*18*), have referred to previous work on the Endophyllum rusts, but it may be well to refer briefly to the conclusions reached by those who have studied nuclear behavior in the spores of rusts, especially forms the aeciospores of which produce promycelia.

Sappin-Trouffy (*27*) was the first to follow nuclear behavior in an aeciospore which in germinating produces a promycelium. He showed that in *Endophyllum euphorbiae-silvaticae* D. C. the mature spore contains two nuclei which, as the spore germinates, migrate, one following the other into the germ tube. They come together side by side as they divide, the two spindles lying parallel in telophase. After the four daughter nuclei have become distributed along the promycelium, two in one end and two in the other, three cross walls are laid down, cutting off one nucleus in each cell. It is all the more remarkable that Sappin-Trouffy should have come to the conclusion that no nuclear fusions occur in this form, since he had found nuclear fusions in every other rust, the germination of the teliospores of which he had studied critically. In Gymnosporangium, Melampsora, Thecopsora, and Cronartium, the fusion nucleus passes out into the promycelium and divides once, the two daughter nuclei moving apart and becoming separated by a cross wall. The two nuclei, though so separated, divide simultaneously and two more cross walls are laid down, completing the four-celled promycelium.

Maire (*14*) undertook a further cytological study of the Endophyllums. He says that in *E. sempervivi* the mature spores contain two nuclei, each with a large nucleole. The nuclei pass into the germ tube, where they divide conjugately (synergiquement), giving the four which are distributed along the promycelium and become separated by cross walls. He notes that *E. sempervivi* "de Gap" has spores that produce long germ tubes; he calls this form var. *aecidioides*, and suggests that it may belong to a long-cycled heteroecious rust. Maire points out that this variety has a great many more spermogonia than the typical *Endophyllum sempervivi* has, and recalls that de Bary had a form of this species of rust on an isolated plant which produced no spermogonia though normal aecidia were developed in abundance.

The situation is somewhat different in *Endophyllum valerianae tuberosae*. The young aceiospore, Maire says, has two nuclei when it is first developed, but one nucleus degenerates so that the mature spore contains only a single nucleus. When the spore germinates this nucleus passes out into the germ tube and is cut off below by a cross wall. Nuclear division follows, and a second cross wall is laid down. A sporidium is formed from the terminal cell only, since the other daughter nucleus degenerates. No spermogonia of this species were ever found by Maire.

Blackman (*3*) followed in some detail the stages in nuclear division in the promycelium from the teliospore of two species of Gymnosporangium. Unfortunately for the writers' particular purpose at this time, he failed to mention when the first cross wall was laid down. His Figure 38 of the two-nucleate promycelium is, in this respect, incomplete.

A form of *Endophyllum euphorbiae* whose aeciospores arise without cell fusions and have only one nucleus from the beginning,

was reported by Moreau (*20*). Other notes on this uninucleate form were published in two later papers (*21, 22*). In a joint paper published with Moreau (*18*) additional information regarding this form is given, and the germination of the spores is described in greater detail. A few stages in the development of the promycelium are figured. The germ tube first contains a single nucleus; this nucleus divides, and later two nuclei separated by a cross wall are seen. The two nuclei divide so that there is nothing in the nuclear behavior in this *uninucleatum* form that differs from the course followed by the fusion nucleus when a teliospore containing a fusion nucleus germinates.

Hoffmann (*9*) was the first to carry on extensive cytological research on *Endophyllum sempervivi*. He had an abundance of material in culture, and apparently exercised great care in studying the details of spore formation and spore development. His best results were obtained by fixing aecia in Juel's solution and staining the preparations with Haidenhain's iron haematoxylin. Since the spores often germinate in the aecium under moist conditions, germination stages were studied from sections only 5 μ thick. Hoffmann says that the nuclei fuse soon after the binucleate spores are dislodged from the chains. This statement is supported by a number of excellent figures showing spores with a sculptured exospore and each spore with a single very large nucleus. Some of the nuclei are in the prophase, others are in metaphase, stages of division. The fusion nucleus does not always wait for germination to take place before it begins to divide. The nucleus usually completes its division before it migrates into the germ tube. One case is shown where the heterotypic division is occurring as the nucleus is about to pass through the germ pore. If a mature spore contains two nuclei they will not have nucleoles, and Hoffmann states that this is further proof that the heterotypic division has already occurred. On this basis he concludes that Sappin-Trouffy probably was mistaken in claiming that there are no nuclear fusions in *Endophyllum euphorbiae-silvaticae*. The second division may also take place occasionally before a spore germinates. Only one figure of a promycelium which affords evidence of a previous nuclear fusion is given in this paper. This figure shows a two-celled promycelium with a nucleus in each cell.

Kunkel followed his preliminary paper (*10*), showing that there is in America a short-cycled form of the blackberry orange rust *Gymnoconia interstitialis*, with a second paper (*11*) on nuclear behavior in the promycelia of this form. He says that the two nuclei in the aeciospore of the short-cycled form fuse so that the mature spore as it is about to germinate will contain only a single nucleus:

> A study of the binucleate spores during germination shows that they become uninucleate previous to the production of promycelia. Preparations made from cultures that were 15 hours old show a large number of uninucleate spores. In some of these preparations more than 90 per cent of the spores contain only one nucleus, thus demonstrating that during the early stages of germination the binucleate spores become uninucleate.

He figures two of these uninucleate spores, one of which has developed a germ tube of some length. He also shows a binucleated spore with a germ tube about the same length, suggesting that in this case the first division has taken place in the spore. The first

division of the fusion nucleus is figured in the diaster stage in the middle of a long germ tube. Although Kunkel saw spores with three or four nuclei, he found no evidence of disintegration of nuclei. He reports promycelia with more than four cells (excluding the stalk), but says that never were more than four sporidia developed in such cases. Kunkel agrees with Hoffmann that Sappin-Trouffy was probably wrong when he reported that there were no nuclear fusions in *Endophyllum euphorbiae-silvaticae.*

Poirault (*25*) described a new species, *Endophyllum centranthi-rubri,* on the red Valerian. The second paper by him (*26*) contains a very interesting summary showing the differences between *E. valerianae-tuberosae* and *E. centranthi-rubri.* Maire (*15*) had questioned whether there was really any difference between the two species. Poirault says he has seen both hosts in the same region, the red Valerian bearing rust but *Valeriana tuberosa* not. Accepting Maire's account of nuclear behavior in *E. valerianae-tuberosae,* Poirault concludes that there are two distinct rusts on these closely related hosts. It is in contrasting the two rusts that he states that the aeciospore of *E. centranthi-rubri* has only one nucleus from the time of its origin. When the mature uninucleate spore germinates, a two-celled promycelium is formed, a single sporidium developing from each cell. Exceptionally three sporidia develop from a promycelium. Poirault discovered in his later investigations that spermogonia were developed on plants which he had infected by sowing spores. He had not previously seen these structures on any of his other collections of this species.

The final paper by the Moreaus (*18*) gives in greater detail the results of their studies reported in preliminary papers (*22, 16, 17*) on *Endophyllum euphorbiae-silvaticae* and *E. sempervivi.* The rust on leaves was fixed in picro-formol, and sections 5 microns thick were stained with Haidenhain's haematoxylin. They found that the nuclei fuse soon after the spores are discharged from the chain and have begun to develop an exospore. If a spore develops four or six nuclei as the result of nuclear division, the supernumerary nuclei degenerate and only two are left to fuse. The spores were germinated on water, floated on glass slides where they were fixed and attached to the slide with collodion, and stained as before. The fusion nucleus undergoes a division in the germ tube, and the daughter nuclei separate and are cut off, one in each cell, by a septum. After the daughter nuclei have rounded up they contain no nucleoles. A second division occurs giving the ordinary four-celled promycelium. The Moreaus agree with Hoffmann as to nuclear fusion in this species, but they seem to differ widely with him in respect to certain important details.

According to Kursanov (*12*), forms of *Aecidium leucospermum* (*Ochropsora Sorbi* Diet.) in which the aecia contain a large percentage of uninucleate spores, arising without cell fusions, are not rare in the vicinity of Moscow. The spores germinate with the regular type of long germ tubes, each having a single nucleus. Just why he was able to infect neither Sorbus nor Anemone with spores of this form is not clear, because, according to him, all aecia of this form contain some binucleate spores.

Two distinct strains of *Caeoma nitens* which are strikingly unlike the short-cycled form reported by Kunkel (*10, 11*) were described

by the senior writer (*5*). It was shown that in one strain no spermogonia are developed, and that most of the aeciospores arise without cell fusions. These spores are uninucleate from their origin, and are very much smaller than are those with more than one nucleus. The uninucleate spores germinate readily, forming a two-celled promycelium, each cell eventually producing only one sporidium. The second strain is very similar to the first, except that it develops spermogonia. Chains of binucleate spores also occur in aecia of both strains. In some cases the basal cell of such chains is binucleate, but certain spores have two nuclei as a result of the division of its original nucleus. The assertion that the two nuclei in binucleate aeciospores of *Caeoma nitens* fuse before the spore germinates is questioned.

Lindfors (*13*) describes an interesting form of *Puccinia arenariae*, in which, he says, the haploid stage has been entirely suppressed. Every cell of the mycelium and of the reproductive structures is binucleate. The fusion nucleus migrates into the promycelium and divides. The spindle elongates and the daughter nuclei are then cut off, one in each cell, by a median septum. The two nuclei then divide simultaneously, and after reconstruction each cell of the promycelium has two nuclei; no other septa are laid down. A single binucleate sporidium develops from each of the two cells. With the germination of the sporidium and the infection of a new plant, the sporophytic condition is continued without a break. No cell fusions occur in the telial sorus, since the hyphal cells are already binucleate.

MATERIAL AND METHODS

Several horticultural varieties of blackberries (Rubus spp.) infected with the short-cycled or *Caeoma nitens* form of *Gymnoconia interstitialis* are grown in the greenhouse and in the field at Arlington Experiment Farm, Rosslyn, Va. Small pieces of leaves bearing aecia in various stages of maturity were killed in Flemming's fixatives. As an additional assurance that fully mature spores were embedded, the method described by Hoffmann (*9*) was also employed. Small sections of agar were cut out of the plate, and pieces of leaves bearing aecia containing large numbers of mature spores were laid in the holes. Melted agar was then poured over the specimens so as to cover the spores in agar. After the agar had solidified, pieces of it containing the specimens were fixed in the usual way. This material was sectioned from 5 to 10 μ thick and stained with the triple stain.

Two distinct methods were used in preparing the material for study of nuclear behavior. The first method of staining the spores was adapted to show their nuclei at maturity and just before germination begins. It consists in coating a No. 0 cover slip with a very thin layer of agar. After dusting the agar with spores, the slip is laid in a damp chamber, the time varying from one to several hours. The spores are then carefully flooded with one of Flemming's fixing fluids. After fixation the preparation is washed and run through the alcohols and stained with acid fuchsin and iodine green. Many of the spores are washed away, but there are always a large number left on the slip. These preparations show the nuclei so clearly that ordinarily the number present can be told with great certainty. Fixation of the cytoplasm and nuclei was very much better if the spores were

allowed to germinate before fixing. This method also proved satisfactory for a study of nuclear migration from the spore into the germ tube.

The second method was especially adapted for a study of germination stages. The spores are germinated on agar in Petri dishes in the usual way, and are allowed to fix in Fleming's fluid for several hours. In order to insure that no spores escape, melted agar may be poured over them, and after this agar has cooled the pieces are fixed as before. Sometimes the fixing agent is mixed with the melted agar, but this method is not fully satisfactory. Sections of this material were cut 20 μ thick and parallel to the surface of the agar. Stained with the triple stain such preparations are highly satisfactory, as they often show the spores with their entire germ tubes or promycelia. For the mature but ungerminated spores to be stained in toto the acid-fuchsin and iodine-green method is superior.

Having selected an infected plant from which material was to be obtained for a cytological study of aecia, series of germination studies were carried out in order that there be no mistake as to the nature of the strain of rust in that particular plant and in the leaf, pieces of which were fixed. Conversely, if a cytological study of mature spores or of promycelia was to be made, it was found very desirable to fix some of the aecia from which spores were obtained, so that a study of the spore chains could be made later.

GYMNOCONIA INTERSTITIALIS, FORM CAEOMA NITENS

The writers have in culture, as noted above, at least four distinct short-cycled strains of the Rubus rust, Gymnoconia. In two strains most of the aeciospores originate without cell fusions and are uninucleate; in the other two strains cell fusions regularly occur, so that most of the spores are binucleate when they are cut off as the spore initial divides. The uninucleate spores of the first two strains develop two-celled promycelia, the binucleate spores of the last two develop four-celled promycelia (*5*).

BINUCLEATE SPORES

The question to be determined first is whether or not the two nuclei originally contained in the spore which is to develop a four-celled promycelium fuse, either as the spore comes to maturity or during any stage of germination. In order to ascertain the facts in this case, the different points in the development and germination of the spores at which such fusions might be looked for have been kept in mind.

Naturally the cytologist looks first of all at the spore chains, following them spore by spore from the basal cells upward. The spores of the short-cycled orange rust are not shot out of the sorus as soon as mature, but tend to cling together and heap up and finally fall out in bunches. There would be an opportunity for nuclear fusions between the time the spores are separated from the chain and the time of their germination, which may even occur in the sorus, especially in moist weather. The longer the resting period of the fusion nucleus the more easily it should be detected. In case of a very short resting period the critical stage might be overlooked, especially if, as Hoffmann (*9*) states for *Endophyllum sempervivi*,

the fusion nucleus divides heterotypically as the spore reaches maturity; the old spores as well as the immature ones would be binucleate.

Fusion might be delayed until germination had actually begun. Two nuclei would be seen in the spore until just before they passed through the germ pore. This would necessitate a critical study of germ tubes which would show a single nucleus at some stage in their growth, if fusion had occurred. When a rust goes over from its long-cycled habit to the short-cycle type, one would expect that nuclear fusions, if they were to occur, would be delayed as long as possible. The last place where such a fusion could be effected would be immediately after the two nuclei had come to rest in the middle of the germ tube. Nuclear divisions would follow at once, so that again the fusion stage might escape notice. Failure to find fusion nuclei at any of the stages mentioned would be adequate proof that no fusions occur, if a detailed account were given of what the nuclei do during these periods. Abundant material was available for study, and no difficulty was encountered in following the course of nuclear behavior step by step from the time the two nuclei come together in the fusion cell until the four sporidia are cut off from the cells of the promycelium.

Examination of hundreds of slides of sections of aecia containing spores in every stage of development, from their origin until the spores are dislodged from the chains and are heaped up in the aecia, fails to disclose the slightest evidence of nuclear fusions up to this time. The paired nuclei of the fusion cell divide conjugately, and the spore initial is cut off. Again there is a conjugate division, after which the aeciospore is cut off with the interstitial cell below. The spore matures and rounds up, and as the exospore becomes distinguishable the two nuclei still remain apart and distinct.

It is the cases where only one nucleus shows in a spore section that compel one to use other methods for following the behavior of the nuclei, because it is often impossible to locate with certainty the same spore in the next serial section. Where spores are stained in toto so that the nuclei show clearly, no such objection can be raised. Preparations of spores stained in toto, after having been allowed to remain under conditions favorable for their coming to maturity and for their passing through the stages preliminary to germination, still show two distinct nuclei. (Fig. 1, pl. 1.) The germ tube begins to appear (fig. 2, pl. 1), and still the two nuclei, each with its nucleole, are clearly visible. As the tube elongates, the nuclei undergo a slight change of form, becoming pear-shaped or pointed, the beak pointing toward the germ pore. (Fig. 3, pl. 1.) The nucleoles, which are always very small in this species, are still visible at this stage, provided the nuclei are well stained. Thousands of spores in these stages stained in toto with acid fuchsin and iodine green have also been studied without any evidence of nuclear fusion having been found. In sectioned preparations of germinating spores, the nuclei are more delicately stained, so that their nucleoles show much more clearly.

The migrating nuclei elongate still more as they enter the germ tube and if fixed at this stage the beaks usually stain heavily, but the pouchlike lower ends maintain their delicate structure. The

peculiar undulating, wavy or S-shaped forms of the drawn-out nuclei (figs. 5, 8, pl. 1) contribute to the orientation of the nuclei in the tube later. Just before the nuclei have completed their passage out of the spore and into the tube they begin to shorten, and the undulating forms which they took while passing through the pore are changed. The process by which two nuclei which migrate side by side through the pore become oriented in the tube, one beyond the other, can be readily understood. (Fig. 6, pl. 1.) As the nuclei pass out of the spore into the tube they appear to be preceded or surrounded above by a light region in the cytoplasm devoid of granular substance and capped by a mass of cytoplasm which stains readily (figs. 6, 8, pl. 1) as though the nuclei were forcing the tube contents outward instead of the nuclei being drawn out, as really may be the case. Either the beak end of one nucleus is slightly in advance of the other (fig. 6, pl. 1) or the outer ends of the elongated nuclei are curved in such a way that one nucleus in its contraction slips by or around and over the other (fig. 11, pl. 1). In shortening and thickening still further, both nuclei become more or less crescent-shaped or arched outward. (Figs. 10–13, pl. 1.) Such a stage, especially if it be poorly fixed, might easily be mistaken for a telophase stage of a heterotypic division.

Although the nuclei often migrate together (figs. 3, 4, 5, 8, pl. 1), or one follows somewhat behind the other (figs. 6, 7, 9, pl. 1), one nucleus may sometimes complete its passage and begin to round up in the tube before the other has entered the germ pore region (fig. 10, pl. 1). Having reached the middle of the elongated germ tube, the nuclei regain their even spherical contour, showing delicately staining chromatin and a distinct nucleole. (Fig. 14, pl. 2.) If the presence of a nucleole in each nucleus is any further proof that nuclear fusion has not yet occurred, we may be sure that no fusion has taken place up to this time, either in the spore or during migration in spore germination.

EXPLANATORY LEGEND FOR PLATE 1

FIG. 1.—Binucleate aeciospore soon after being freed from the chain.

FIG. 2.—Early stage in spore germination. The nuclei show large nucleoles deeply stained with acid fuchsin.

FIG. 3.—Later stage in spore germination. One nucleus elongated, directed toward the germ pore, the other showing nucleole distinctly. The length of the germ tube is about the diameter of the spore. Cytoplasm of the spore becoming vacuolate.

FIG. 4.—Slightly later stage. The beak of one nucleus entering the germ pore. Other nucleus somewhat elongated.

FIG. 5.—Nuclei showing chromatin in a reticulate condition, the beaks deeply stained. Nucleole visible in one nucleus.

FIG. 6.—Nuclear beaks entering the germ tube, and showing granular chromatin mass. Migrating nuclei surmounted by a more densely stained cap of cytoplasm, each nucleus showing a distinct nucleole.

FIG. 7.—Stage somewhat similar to the preceding, except that one nuclear beak overlies the other and is somewhat curved at the end.

FIG. 8.—Nuclei, proceeding together through the germ pore. They are drawn out into S-shaped figures, surrounded by a clear area in the cytoplasm of the germ tube. Cytoplasm above the nuclei more deeply stained.

FIG. 9.—Nuclei migrating from the spore, one in advance of the other. The outer one surrounded by a hyaline area, and more densely stained.

FIG. 10.—Later stage of another type of migration but similar to the preceding, one nucleus having reached the middle of the germ tube, the other, much elongated, passing through the germ pore.

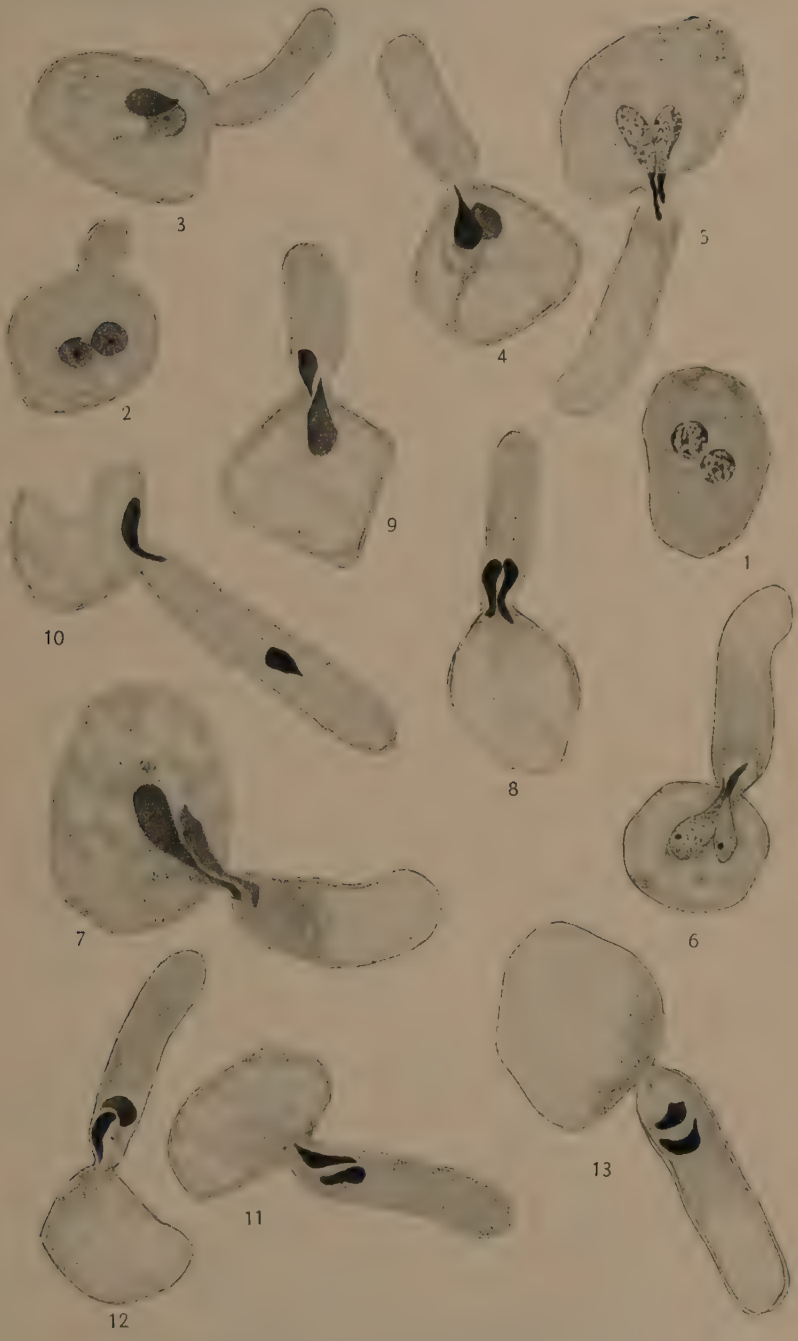
FIG. 11.—Shows how one of the two nuclei which pass through the germ pore together slips by the other and rounds up, the two coming to lie in the germ tube one beyond the other.

FIG. 12.—Might be interpreted as being somewhat earlier stage than figure 11. Shows how the elongated and curved nuclei slip by one another in becoming oriented in the germ tube.

FIG. 13.—Two nuclei completely oriented and beginning to round up. Both somewhat crescent shaped.

(All figures were drawn with the aid of a camera lucida, Zeiss 2 mm., 1.40 aper. and 1.5, 1.30 aper. apochromat lenses, and 8 comp. oc. Nos. 1, 5, and 6, are from material sectioned 20 μ thick and stained with Flemming's triple stain. Nos. 2, 3, 4, 7, 8, 9, 10, 11, 12, and 13, are from material stained in toto, with acid fuchsin and iodine green.)

(The exospore is not shown in any of the figures.)



Stages in nuclear migration in the germination of binucleate spores
(For explanatory legend see page 1010)

Stages in nuclear division of binucleate aeciospores
(For explanatory legend see page 1011)

The possibility that the nuclei might fuse as they lie in the middle of the promycelium has not been overlooked, although, to the writers' knowledge, no one has ever reported such a phenomenon in the rusts. This stage has been studied most critically without finding any suggestion that such a thing ever occurs here. The nuclei in this stage may be fixed so that their chromatin strands stain most delicately. Following the stage in which the chromatin appears finely reticulate (fig. 14, pl. 2), as is usually found in resting nuclei, the network becomes heavier, and more deeply-staining granules are distributed along the threads (fig. 15, pl. 2). A little later more evenly staining strands of chromatin of different lengths appear to be somewhat peripherally arranged in the nucleus, leaving much of the nuclear region clear and unstained. (Fig. 16, pl. 2.) In one case the chromatin strands were clearly polarized, and at least 14 loops were counted. (Fig. 42, pl. 4.) In the metaphase stage and very early anaphase stage the spindles are certainly very short. (Fig. 17, pl. 2.) The two nuclei may and do most often divide simultaneously, but they do not necessarily divide conjugately. The spindles in the metaphase may lie one ahead of the other (fig. 18, pl. 2), so that they are not exactly side by side, although they may become so in the telophases (fig. 20, pl. 2). One nucleus may complete its division while the other is still in the anaphase. (Fig. 17, pl. 2.) Figure 19, Plate 2, shows again one nucleus in a more advanced stage of division than the other, which is apparently in an anaphase stage, the chromatin moving along the spindle fibers. The upper nucleus shown in Figure 18 (pl. 2) is in a slightly later stage of division, when most of the chromosomes have reached the poles, but some are still lying along the spindle. A distinct cone of fibers is seen in the lower nucleus of this figure, again suggesting a short spindle comparable to that shown in Figure 30, Plate 3.

When the chromatin masses have reached the poles and begin to separate, they are still connected by a strand or strands of fibers. (Fig. 20, pl. 2.) It is soon after this stage that one spindle slips by the other, a pair of daughter nuclei going to each end of the promycelium. Figures 21, 22, and 23 (pl. 2) show successive stages of the nuclear distribution. No vestige of fibers or strands connecting sister nuclei is evident in Figure 23, where one pair of sister nuclei has completely slipped ahead of the other. A similar stage is shown

EXPLANATORY LEGEND FOR PLATE 2

FIG. 14.—The germ tube more elongated. The nuclei completely rounded up, and lying one beyond other, and not side by side. Nucleoles showing plainly, lying in a rather distinctly stained granular chromatin.

FIG. 15.—Chromatin masses showing clearly in early prophases of division.

FIG. 16.—Chromatin masses in bouquetlike arrangement of chromatin threads.

FIG. 17.—One nucleus has completed the division. The other shows delayed division, probably early anaphase.

FIG. 18.—One nucleus in early anaphase, the other in telophase; a number of distinct fibers extend between the two polar masses of chromatin.

FIG. 19.—Both nuclei undergoing division. One in telophase, with fibers still stretching between the polar masses, the other the chromatin bodies still scattered along the spindle.

FIG. 20.—The nuclei dividing, their spindles lying almost parallel in the promycelium.

FIG. 21.—Late stages in nuclear division, one spindle slightly ahead of the other.

FIG. 22.—Late stages in nuclear division, one spindle lying at an angle in the bent promycelium.

FIG. 23.—Nuclear division nearly completed. The spindles with their reorganizing pairs of nuclei have slipped by each other, and the four daughter nuclei will come to lie in a line along the promycelium.

FIG. 24.—The pairs of sister nuclei reorganized and separated in the promycelium by the median cross wall.

(All figures were drawn with the aid of a camera lucida, Zeiss 2 mm., 1.40 aper. and 1.5, 1.30 aper. apochromatic lenses, and 8 comp. oc. All drawings on this plate are from material sectioned 20 μ thick, and stained with Flemming's triple stain.)

(The exospore is not shown in any of the figures.)

in Figure 44 (pl. 4), where there is no doubt as to the relation of the nuclei. Ordinarily a septum is laid down as the nuclei of the pairs are becoming reorganized, so that one is certain that the two end cells possess sister nuclei. (Fig. 24, pl. 2.) The two sporidia formed at one end after the laying down of the cross walls will be the direct descendants by vegetative division of one of the two nuclei entering the fusion cell at the origin of the spore chain. The two sporidia at the other end will be alike, having exactly the same genetic constitution as the other nucleus which entered the fusion cell. There has been no nuclear fusion, no reduction division, and therefore no chance for a mixing and recombination of hereditary elements.

UNINUCLEATE SPORES

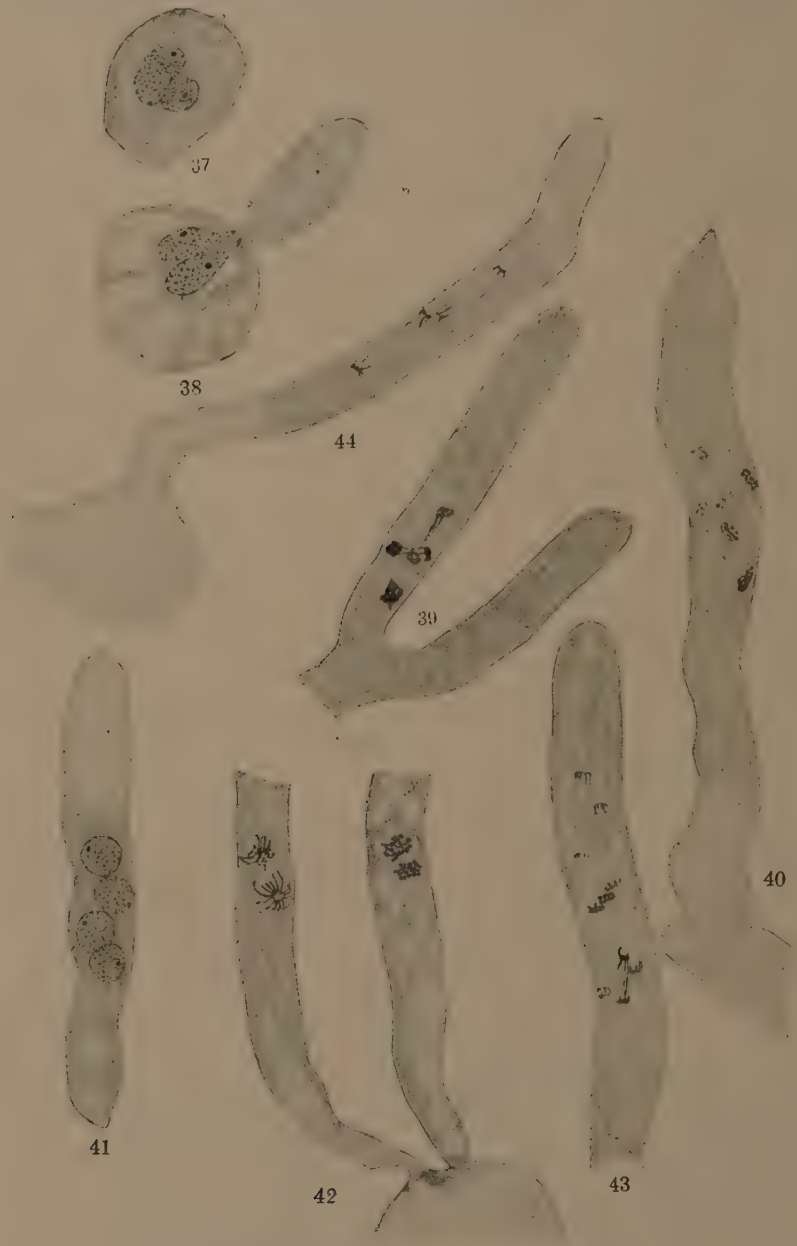
Having followed the binucleated spores from their origin up to and through their germination stages without finding any evidence of nuclear fusion, it is easy to understand what happens if for any reason a polynucleate or a uninucleate spore is developed. The germination of uninucleate aeciospores and the formation of two-celled promycelia by such spores was described in an earlier paper by Dodge (*5*), but the cytological details in the migration and division of the spore nucleus had not at that time been fully worked out. Recent studies of this form show that while a binucleate or polynucleate spore may arise by nuclear division in a chain, the basal cell of which has only one nucleus, the writers suspect that ordinarily after a spore has been dislodged from the chain no further division takes place in the spore. No cases of nuclear division have as yet been found within such spores as they are about to germinate or have actually begun the process. The single nucleus elongates into a beak (fig. 25, pl. 3), which passes through the germ pore with the pouchlike lower part following.

When well into the germ tube, the nucleus shortens and becomes crescent-shaped, meanwhile passing out into the middle of the tube where it will round up, still showing its nucleole. A septum may be laid down at this time, cutting off the outer end of the tube with the single nucleus. The single nucleus then passes through prophase stages of division (fig. 27, pl. 3) similar to that noted for each one of the pair of nuclei (fig. 15, pl. 2). In Figure 28, Plate 3, is shown what is apparently a lateral view of an equatorial plate arrangement of the chromosomes, while Figure 29, Plate 3, is probably a somewhat

EXPLANATORY LEGEND FOR PLATE 3

FIG. 25.—The single nucleus which is drawn out into a sharp beak shows a distinct nucleole.
FIG. 26.—Much elongated nucleus passing through the germ pore, surrounded by a hyaline area in the cytoplasm.
FIG. 27.—The nucleus has passed out to the middle of the promycelium, and is now in early stage of division similar to that shown in Figure 15 of the binucleate aeciospore.
FIG. 28.—Lateral view showing the chromosomes arranged along the equatorial plate.
FIG. 29.—Rather oblique view of chromosome masses in equatorial plate.
FIG. 30.—Later stage in nucleus division. Chromosome masses on a distinct spindle, which is more highly enlarged in Figure 30A.
FIG. 31.—Somewhat later stage. Some of the chromatin masses have already reached the poles.
FIG. 32.—The chromatin has gone to the poles, the spindle fibers still persistent.
FIG. 33.—Late telophase, distinct fibers stretching between the polar masses. End of germ tube cut off.
FIG. 34.—Daughter nuclei completely separated and reorganized. Long, delicate fibers still visible.
FIG. 35.—The sister nuclei reorganized and separated by a cross wall in the promycelium, the outer apical cell elongating in preparation for the formation of a sporidium.
FIG. 36.—Mature promycelium, showing two uninucleate sporidia.
(All figures were drawn with the aid of a camera lucida, Zeiss 2 mm., 1.40 aper. and 1.5, 1.30 aper. apochromatic lenses, and 8 comp. oc. All drawings on this plate are from material sectioned 20 μ thick and stained with Flemming's triple stain.)
(The exospore is not shown in any of the figures.)

Nuclear migration and division in uninucleate aeciospores
(For explanatory legend see page 1012)



Nuclear behavior during germination of multinucleate aeciospores
(For explanatory legend see page 1013)

oblique polar view. The chromosome bodies are so small and closely crowded that the number drawn is probably only an approximation of the number actually present. Figure 30A, Plate 3, which is the spindle of Figure 30 enlarged, shows a very definite spindle, though the chromosomes seem to be already moving toward the poles. It is possible that the stage showing a definite equatorial arrangement does not persist long, since it has been found so rarely, whereas telophase stages are very common. Nuclear division occurs, and the polar masses become separated (figs. 31–34, pl. 3) and show the connecting fibers. After reorganization the second septum is laid down (fig. 35, pl. 3) and the sporidia will be developed (fig. 36, pl. 3).

POLYNUCLEATE SPORES

Turning to the polynucleate spores (fig. 37, pl. 4), it will be found that the behavior of each of the nuclei is precisely the same as it would have been if the spore had been provided with only one or two nuclei instead of three or more. In one spore, for example, three nuclei were clearly seen migrating together; in another spore one nucleus had completely passed into the tube and had rounded up, while the other two were just entering the pore. (Fig. 38, pl. 4.) Having reached the middle of the promycelium, the three, four (fig. 41, pl. 4), or more nuclei round up, each showing a nucleole. Nuclear division then proceeds by the formation of spindles and the division of each nucleus into two daughter nuclei, as was found for the single nucleus of a uninucleate spore, or each of the nuclei of a binucleate spore.

The promycelium from a polynucleate spore is very likely to be deformed or abnormal. In some instances two separate germ tubes are formed from the same spore, each taking a pair of nuclei. (Fig. 42, pl. 4.) One of the two nuclei in the left tube distinctly shows 14 chromatin loops in a large clear nucleus, and is probably a prophase stage comparable to that of Figure 16, Plate 3. Figure 39, Plate 4, shows a branched promycelium with all three dividing nuclei still in one arm. Stages are readily found showing three (fig. 40, pl. 4), four, or five (fig. 43, pl. 4) spindles arranged at various angles to each other and lying about in the broad tube. It is clear that the

---

EXPLANATORY LEGEND FOR PLATE 4

FIG. 37.—A three-nucleate spore just beginning to develop a germ tube. Nucleoles showing clearly in the granular chromatin.

FIG. 38.—A later stage in the germination of a three-nucleate spore. Two nuclei elongated and showing beaks proceeding through the germ pore. The third nucleus, already in the tube, shows a distinct nucleole.

FIG. 39.—A branched promycelium, showing the three nuclei undergoing division. In the lower nucleus the chromosomes are still at the equatorial region of the spindle. The other two are in more advanced stages of division, their axes being at right angles to each other.

FIG. 40.—Three nuclei have already completed their division. Delicate fibers between two of the pairs are seen.

FIG. 41.—Four nuclei as they lie in the promycelium after they have migrated out of the spore. This stage comparable with that shown in Figure 14, Plate 2, of the binucleate aeciospore.

FIG. 42.—A rather unusual type of germination of a four-nucleate aeciospore, two nuclei showing in each of the separate germ tubes. The nuclei in the promycelium at the left show a boquetlike arrangement of the chromatin threads.

FIG. 43.—Five nuclei completing division. The two corresponding polar masses from each of the dividing nuclei can be recognized by careful focusing.

FIG. 44.—The two spindles of a division of a binucleate spore slipping by one another. This figure was obtained from material which regularly shows uninucleate aeciospores.

(All figures were drawn with the aid of a camera lucida, Zeiss 2 mm., 1.40 aper. and 1.5, 1.30 aper. apochromatic lenses, and 8 comp. oc. All drawings on this plate are from material sectioned 20 μ thick and stained with Flemming's triple stain.)

(The exospore is not shown in any of the figures.)

nuclei are not all in the same stages of division. In Figure 39, Plate 4, the chromosomes of the lowest nucleus are still arranged equatorially on the spindle, while those of the other two nuclei have nearly all reached the poles. Such cases of the delayed division of one nucleus in the promycelia of binucleate spores are shown in Figures 18 and 19, Plate 2. All evidence supports the view that each nucleus in a spore is capable of independent and individual action during migration and division. Binucleate spores are found occasionally in preparations from aecia containing mostly uninucleate spores. Figure 44, Plate 4, represents such a case. This figure furnishes further evidence of the manner in which one spindle slips by the other. (Figs. 20 to 23, pl. 2.)

The number of cells in a promycelium is largely determined by the number of nuclei originally present in the mature spore. The particular form of the promycelium, whether it be double, branched, or lobed, is not accounted for readily, but the number of cells and sporidia it will develop depends on the accidental distribution of the pairs of daughter nuclei. Dying of certain cells or degeneration of certain nuclei may occur, but if such things do not happen and all goes well there will be eventually twice as many sporidia on a promycelium as there were nuclei in the mature spore.

DISCUSSION

Evidence has been presented to prove that there are no nuclear fusions in the life of the short-cycled *Caeoma nitens* strains of the Rubus rust. It has been held constantly in mind that it would be necessary to follow the pair of nuclei step by step from the time they first come together in the fusion cell until they divide and become separated by a cross wall in the promycelium.

A nuclear fusion in the teliospore has come to be regarded as the one constant and most essential process in the life of every rust. It is now 30 years since Sappin-Trouffy (*27*) was so bold as to assert that there are no nuclear fusions in *Endophyllum euphorbiae-silvaticae*. Even though the Moreaus have proved beyond a doubt that Sappin-Trouffy was right, the significance of his discovery is yet to be appreciated. This part of his work is not mentioned even by either Kursanov or Lindfors. This attitude may be due to the fact that most students of the rusts have been more particularly interested in finding out about how the two nuclei get together in the basal cell, with the result that the phase of the question dealing with what becomes of them later has been somewhat neglected. What a different reaction was stimulated by Hoffmann's (*9*) announcement that the two nuclei in the aeciospores of *E. sempervivi* fuse as the spore comes to maturity. The statement is always referred to and accepted without question because it was what everyone expected. In his discussion suggesting the possibility that Endophyllum is a reduced form and not primitive, Fromme (*6*) says: "The assumption of the teleutosporic method of germination by an aecidiospore is not a difficult conception, since nothing more than the fusion of nuclei in the spore should be necessary for its accomplishment." *E. euphorbiae* and *Caeoma nitens* are clearly reduced forms, and they became such even without nuclear fusions.

After all the years of cytological work dealing with nuclear fusions, reduction divisions, pollen tetrad formation, and other phases of the cycles associated with fertilization in green plants, as well as fungi,

it is not easy to imagine that there could be developed normally by a rust a four-celled promycelium with four sporidia as a result of "sexual" fusions without there having been a reduction division. Yet that is what the discovery by Sappin-Trouffy means, and the discovery of the same course of development in *Caeoma nitens* means that there are probably other similar cases yet to be brought to light.

It is a fact exemplified many times over in the evolution of plants and animals that the factors that stand for or represent a morphological structure are not indissolubly connected with those factors that determine how the structure shall function. If an organ ceases to perform its original function and takes on a new function, or takes over work performed heretofore by another organ which has been suppressed in evolution, the scientist may give the organ a new name which will more nearly connote the change of function. He never forgets, however, what that organ represents in phylogeny, neither does he apply to it a name by which the suppressed organ is known. When a long-cycled rust like *Gymnoconia interstitialis* becomes short-cycled not only by suppressing the telial stage but also by omitting the most essential physiological activity consummated heretofore in the teliospore, the fact is not explained by referring to the case as teratological or as a monstrosity. Promycelia which develop seven or eight sporidia need no longer be called abnormal, because it is now known that these multicellular promycelia come from multinucleate spores in which no nuclear fusions occur. The factors representing septate germ tubes or promycelia and sporidia are active in *Caeoma nitens*. There occurs a pseudofertilization in the cell fusions without an ultimate nuclear fusion, and this is followed by a pseudoreduction by cell division in the promycelium.

According to all the evidence to date, the nuclei in the auxiliary cells of the Florideae take no part by way of nuclear fusions in the inauguration of spore production, yet these same auxiliary cell fusions play the leading rôle, or are the first essentials, in the development of carpospores in all but the simplest of the red algae. The nuclei in the ooblastema filaments are diploid in consequence of a previous sexual union between sperm and egg. A fusion between a diploid and a haploid nucleus would not be expected in connection with the fusions between auxiliary and ooblastema cells. The rusts may have evolved from the red algae with the loss of the carpogonial branch. In those species where the auxiliary cells are arranged more or less compactly in a palisade, the opportunity for cell fusions between haploid cells would be afforded. The first rusts originating at this time may have completed their life cycles without nuclear fusions, and only later become so adjusted to the new state as to bring to bear the full effect of the auxiliary cell fusion by a nuclear fusion. Cell fusions in the rusts are not sexual fusions, and the cells involved are not sex organs any more than are the anastomoses of hyphae involved when the + and − strains of a heterothallic Coprinus come together. The effects of the cell fusions are just as potent as sexual fusions and perhaps even more so; the substitute process is not necessarily inferior. The Ascomycetes may very well have been evolved from the red algae when the tip of a diploid ooblastema filament bent about and fused with its subterminal cell. With a fusion of two diploid nuclei and a double reduction as proposed by Harper (*8*) the eight

nuclei in the ascus would be accounted for. It is not necessary that a double fertilization including two separate nuclear fusions should occur in every ascomycete to-day. It is conceivable that the inheritance handed down originally from the fusion of diploid nuclei is still manifested in the three divisions in the ascus, even when no nuclear fusions occur. The promycelia of *Caeoma nitens* and *Endophyllum euphorbiae* have four cells in spite of the fact that there have been no nuclear fusions and no reduction divisions.

It is obvious that one can not deny nuclear fusions because he finds in stained preparations two nuclei in every mature spore, unless a large part of the original sowing of the spores is allowed to complete germination and it is found that promycelia and not long germ tubes develop in these controls. On the other hand, one can not maintain that nuclear fusions occur on the basis of finding a large percentage of mature spores with a single nucleus in each spore, unless an equally large percentage of spores in control cultures develop four-celled and not two-celled germ tubes.

The long-cycled and short-cycled Rubus orange rusts grow side by side on blackberries. In certain strains both long-cycled and short-cycled aeciospores are sometimes developed in the same sorus. It is just as essential to provide checks or control cultures in this cytological work as it would be when carrying on infection experiments. This is a relatively easy procedure where spores are dusted on agar in Petri dishes or on cover slips coated with agar. As noted previously, germination studies have been carried on to check all the material used for the cytological studies upon which this paper is based.

In discussing types of promycelia produced by strains of Endophyllum or *Caeoma nitens*, it is assumed that one is referring to germ tubes developed under conditions such that the nuclear processes can go on normally. This means that spores should be germinated as soon as shed naturally. They should be floated on water in moist chambers or dusted on the surface of water agar, and kept in a cool place. Germ tubes that grow under water or down into the agar are not normal, as is generally known.

Sections of telia of the Gymnoconia strain on *Rubus occidentalis* show that nuclear fusions do occur in the teliospore of this form. Any stained preparation of a fairly young sorus shows that the spores are originally binucleate, and that the nuclei fuse very early. An exhaustive search is not necessary to convince one of the facts in this case. The diploid nucleus is only slightly larger than are the haploid nuclei which fuse, enough difference, however, to allow for the fusion, since the volumes would vary as the cubes of their diameters.

It has been pointed out that in *Endophyllum sempervivi* there is a strain sometimes found in France which develops long-cycled true aeciospores. Maire (*14*) has suggested that this strain may belong to a heteroecious long-cycled rust, since he was unable to reinfect the Sempervivum with the spores of the variety *aecidioides*. Disregarding for the moment the obvious fact that the complete story of nuclear behavior in this species has not all been told, and accepting the view that at least in one strain of the rust there are nuclear fusions, it will be asked why there should not be found similar races in *Caeoma nitens* where the nuclei fuse. In addition to the long-cycled form there are certain strains in which the aeciospores are

very regularly binucleate, and their promycelia are very constantly four-celled, so that exceptions to this rule are hard to find. It is in such strains that nuclear fusions should be found if they ever occur. Strains of this type particularly have been studied without the discovery of any evidence of nuclear fusions. Their spores germinate just like they do in *E. euphorbiae-silvaticae*. If the mature spore has two nuclei, the promycelium will be four-celled. The two nuclei entering from the spore divide simultaneously, and it is not until the pairs of sister nuclei have slipped by each other that the pairs become separated by a cross wall. One never finds in this form a two-celled promycelium with one nucleus in each cell. However, uninucleate strains with spores which have arisen without cell fusions produce two-celled promycelia with only one nucleus in each cell. Polynucleate spores give rise to the so-called abnormal germ tubes with variable numbers of cells, depending upon the food supply available and upon the accidents occurring during the distribution of the nuclei.

According to Sappin-Trouffy (*27*) and Dodge (*4*), when the aeciospores of certain species germinate, a septum is often laid down, cutting off that part of the germ tube containing most of the cytoplasm. The empty part of the tube next to the spore may look like a stalk, but it has no such morphological significance. The older and longer the germ tube becomes the more likely will such a septum be found. It is a device for confining the living contents of the cell in a smaller inclosure. Kunkel (*11*) says that even if a promycelium of *Caeoma nitens* has five or more cells (exclusive of what he calls the stalk cell), only four sporidia are developed. This would be natural if a nuclear fusion occurred. The writers have seen any number of cases where more than four sporidia were developed on the same promycelium. The absence of nuclear fusions contributes to the perfect functioning of a spore, no matter how many nuclei it may contain at maturity. The so-called abnormal promycelia with three, five, or more cells are not found in the strains of *Caeoma nitens* whose spores are regularly binucleate when mature.

Moreau's *Endophyllum uninucleatum* is a most unique form of rust. The spores arise without cell fusions and germinate just like a teliospore possessed of a fusion nucleus, producing four-celled promycelia. They go through the form of reduction completely, two divisions and four daughter nuclei. Moreau, who has examined preparations of the uninucleate form of *Caeoma nitens* showing germinating spores and two-celled promycelia, says in a letter to the writers that it is entirely unlike their uninucleate Endophyllum. The Moreaus (*18*), in commenting on Poirault's uninucleate Endophyllum on the red Valerian, say it is exactly like their uninucleate form. Such a conclusion is clearly not warranted, because Poirault (*26*) says distinctly that in his rust the promycelium is regularly two-celled and only two sporidia are developed whereas in *E. uninucleatum* it is claimed the promycelium is four-celled. It should be pointed out, however, that in no place have the Moreaus figured a promycelium of *E. uninucleatum* bearing four sporidia. The binucleate spores of *E. euphorbiae-silvaticae* are even slightly smaller than are those of the *uninucleatum* form. If the latter strain of rust has been derived from the former by simply omitting the cell fusions, it is strange that its spores should be so large. Still more difficult is it to explain why the nucleus should divide twice during germina-

tion, when the nuclei of its parent type divide only once. In *E. centranthi-rubri* the nucleus likewise divides only once during germination. It is certainly interesting to find these cases where, though no nuclear fusions occur in the life cycle, a nucleus goes through the form of reduction so meticulously.

A spore may be uninucleate at its origin, and yet contain several nuclei by the time it is set free. The writers have found that the order of nuclear division and the laying down of cross walls in a germ tube is indicative of the nature of the aeciospore producing it. If the promycelia are slender and have two cells, the spores in the control cultures, if fixed before germination begins, will show only one nucelus when stained. Promycelia with seven or eight cells come from giant polynucleate spores. In *Endophyllum sempervivi* one could tell by the same criterion that the aeciospores had a single nucleus, but he could not tell whether the spore contained a fusion nucleus or had a single nucleus from the time of its origin. The same rule would hold true for *E. euphorbiae-silvaticae*. If a promycelium at maturity has only two cells, and a sporidium is developed from either one or both the cells, the writers hold that the spore from which it developed originally contained a single nucleus. On this basis the two-celled promycelia bearing sporidia figured by the Moreaus (*18*, *p. 19*, *fig. 2*) would be interpreted as showing that the spores were originally uninucleate rather than binucleate, as they state. Whether or not the various stages in the development of promycelia in the rusts is correlated throughout the group with nuclear behavior remains to be determined. In cases so far described, when a teliospore containing a fusion nucleus germinates, the median septum is laid down in the promycelium before the second nuclear division is well under way. All such promycelia have a two-celled stage with one nucleus in a cell. The writers believe this to be the best evidence presented by Hoffmann (*9*) and the Moreaus (*18*) for nuclear fusions in *Endophyllum sempervivi*. In cases where a spore which is to form a promycelium has only one nucleus, haploid or diploid, there will then be a two-celled stage of the germ tube with only one nucleus in each cell, so in the early two-celled stage it might be difficult to tell, as noted above, whether the spore had a haploid or a diploid nucleus at the time of germination.

The Moreaus (*18*, *19*) seem to have been the first to follow the nuclear behavior in those abnormal aeciospores which contain three or more nuclei. They may be correct in their explanation of their disappearance. Degeneration of supernumerary nuclei is not uncommon. Hoffmann (*9*), however, figures promycelia of the same species with eight sporidia. If no nuclear fusions occurred this could be readily understood. If it is admitted that there can be a fusion of nuclei in pairs in the aeciospore the eight sporidia are also easily accounted for.

Kunkel's Figure 8 (*11*) shows very well the two spindle figures in the promycelium after they have slipped by each other. This could not represent the second divisions of a fusion nucleus as he states, unless it were in a very different type of promycelium development than has been commonly described. After the first division of the fusion nucleus in the germ tube from teliospores and from aeciospores of *Endophyllum sempervivi*, as noted above, a septum is formed between the two daughter nuclei.

In multinucleate cells generally such as oogonia, ascogonia, and asci, and in plasmodia, there is a tendency for all of the nuclei occupying the same cytoplasm to divide at the same time. The crosier or ascus hook, and the clamp connections of the higher basidiomycetes, are admirable devices in the development of which nonsister nuclei are cut off in the new cell after a conjugate nuclear division. The two nuclei in the fusion cell and in the spore initial of the rusts divide conjugately, and the presence of nonsister nuclei in the spore is thus assured. In *Caeoma nitens* and *Endophyllum euphorbiae-silvaticae* the nonsister nuclei that come to lie at the center of the promycelium do not divide conjugately in the full sense of the word as it is now used. Conjugate division now also connotes the cutting off of nonsister nuclei in the new cell, whereas in the two cases just mentioned sister nuclei come to occupy the same cell for a short time or until they are separated by a cross wall. A promycelium from a giant spore will contain several nuclei which may divide simultaneously, but their spindles do not all lie parallel; on the contrary, they may extend in any direction, especially as such promycelia are likely to be rather broad and somewhat branched or irregular in shape.

Since announcement of the discovery that strains of *Caeoma nitens* which have no spermogonia have uninucleate aeciospores (*5*) many further observations have been made, all of which confirm the account given at that time. Beyond the suggestion that the behavior of the rust as it exists in certain plants infected artificially is such as to raise the question of heterothallism, the writers have no further explanation to offer regarding the absence of cell fusions regularly in strains without spermogonia. De Bary (*2*) noted the absence of spermogonia on an isolated plant infected with *Endophyllum sempervivi*, while Maire (*15*) pointed out that the variety *aecidioides* always had a greater abundance of spermogonia than he ever found on the short-cycled type. Maire probably never saw spermogonia of *E. valerianae-tuberosae*. Poirault (*26*) says that it was two years before he was able to find spermogonia on leaves infected with *E. centranthi-rubri*. He thinks that if Maire had made a further search, perhaps he would have found spermogonia in the first species. Both might be right. There very likely are strains which, though possessing uninucleate aeciospores, develop spermogonia regularly, and other strains with uninucleate spores that do not develop spermogonia. Maire says that the single nucleus passes through the pore into the germ tube, but he does not show this. The nucleus divides, and a cross wall is laid down. The lower cell loses its nucleus by karyohexis, or it may divide and then both daughter nuclei degenerate. If the story of what the nuclei do in the aeciospore as it matures and germinates is as clear as the reader may suppose from Maire's description, it would have been more convincing if he had figured some of the real critical stages. It is possible that in some of the material of *E. valerianae-tuberosae* studied by Maire the spores have only a single nucleus from the beginning, just as Poirault claimed for his Endophyllum on a closely related host. If one of the two nuclei in the two spores degenerates, the formation of a two-cell promycelium such as he figured is readily understood when one recalls the promycelium formed by *E. centranthi-rubri* and the uninucleate *Caeoma*

nitens. Maire may be correct in his description of nuclear behavior in *E. valerianae-tuberosae*, but the account certainly needs confirmation.

Olive and Whetzel (*24*) were unable to account for the production of only two spores on the promycelia of *Endophyllum stachytarphetae* and *E. circumscriptum*. A cytological examination of the aecia would no doubt be sufficient to enable one to say whether this was due to the lack of cell fusions as in other uninucleate Endophyllums, or whether, as Lindfors (*13*) claims for *Puccinia arenariae*, the promycelia remain two-celled, even after the second division and two sister nuclei pass up into each sporidium.

In the higher basidiomycetes, such as Coprinus, heterothallism is not a generic peculiarity, although with perhaps rare exceptions it is specific. Absence of spermogonia in *Caeoma nitens* as well as in certain of the Endophyllums may always be correlated with the absence of cell fusions and the production of two-spored promycelia. Other species of Endophyllum may have binucleate aeciospores, as Olive and Whetzel's account also seems to infer, without there being spermogonia; only one of the four species which these investigators studied in Porto Rico had these structures. It also would be interesting to know how the teliospores of *Uromyces rudbeckiana* noted by Olive (*23*) would have germinated. He says this species presented an enigmatical variation, in that all the cells—teliospores as well as the vegetative mycelium—had but one nucleus.

Kursanov (*12*) was unable to infect either Anemone or Sorbus with spores from the strain of *Aecidium leucospermum* having mostly uninucleate aeciospores. He recalls, however, that the possibility of a repeated infection of Anemone by this fungus has been demonstrated by Soppitt (*28*), who obtained the development of aecia a year after inoculation. Kursanov (*12*) suggests that perhaps the frequent existence of uninucleate aeciospores may explain this repeated development of aecia on the first host of a heteroecious rust. While Kursanov admits that it would be interesting from a theoretical standpoint to know the cycle of development of such somatically apogamic rusts as *Endophyllum uninucleatum* (*20*), Olive's (*23*) *Uromyces rudbeckiana*, and the uninucleate forms which he had discovered, yet he thinks such fungi should be regarded as accidental monstrosities without profound significance. Opposed to such a view, it might be said that not only would some of the most puzzling questions from a practical standpoint be answered, but the scheme of rust evolution would be made clearer, if all these "monstrosities" were better understood.

Barclay (*1*) showed that repeating aecia are produced when aeciospores of *Uromyces cunninghamianus* are sowed on the host Jasminum. Barclay's account of this fungus is of special interest. The teliospores are developed in the old aecial cups; spermogonia occur only following infection by sporidia from teliospores. According to Barclay, no such structures are found among the secondary aecia. Just how typical or normal for the species is the mode of aeciospore germination described by Barclay is hard to say. He suggests that his fungus may have certain relationships to the Endophyllums, principally because the aeciospore produces a two-celled promyceliumlike germ tube. Each cell puts out a sterigmatous

branch by means of which, he thinks, the host is infected. No sporidia were ever seen on these long sterigmata. Branched germ tubes from aeciospores and uredospores of several species of rusts have been figured (*27*, *4*). The two nuclei in the tube divide precociously, and the cytoplasm is cut off in the outer end by a single septum. In these cases the germ tubes are long and slender. Those of Barclay's Uromyces are short, comparatively thick, and very much like certain two-celled promycelia of an Endophyllum (*20*, *2*), or the uninucleate *Caeoma nitens* (*5*)[3] which failed to develop sporidia because of cultural conditions or age of the spores. The failure of the sterigmatous branches to produce sporidia is not proof that the two-celled structures of Barclay's fungus are not real promycelia. The life history of this rust is no doubt more complicated than at first appears.

Presumably Lindfors's (*13*, *p. 44*, *fig. 17*) text figure of two-celled mature promycelia of *Puccinia arenariae* was drawn from the same gathering of the rust which he fixed for cytological study. His account of nuclear behavior seems to be conclusive, except at one critical point, viz, he does not show that the two sister nuclei which he finds in each cell actually migrate into the sporidium. Heretofore, whenever a sporidium has been found to contain two nuclei, it has been assumed that the original nucleus of the sporidium had divided. Grove (*7*), as Lindfors pointed out, figured four-celled promycelia for this species. Grove says: "No doubt the future will find this Puccinia divided into several biological races if not into distinct species." Wolf (*29*) has described the development of ascospores of *Podospora anserina*. The ascus has regularly eight nuclei, but in the delimitation of the spores two nuclei are included within each spore membrane. The ascospores of this fungus and the sporidia of *Puccinia arenariae* become binucleate in an analagous manner. The production of only two sporidia on the promycelia of the Endophyllum referred to by Olive and Whetzel may also have been due to a similar process of development.

Endophyllum euphorbiae and *Caeoma nitens* have much in common. Both are systemic, perennial, short-cycled rusts. Their binucleate strains have exactly the same nuclear behavior throughout, and produce the same types of promycelia; both have uninucleate strains. Nevertheless, the two rusts may be widely separated in their phylogeny. It is their general morphological character, rather than their habits of parasitism or nuclear behavior, that points the way of phylogeny.

Two binucleate races of *Caeoma nitens* seem to differ in the size, color, and form of their aecidiospores, and one race seems to have more numerous and more prominent spermogonia. Their sporidia may differ in size, although this feature has not been sufficiently studied to warrant a positive statement on this point.

The two uninucleate races differ at least in one respect. One race develops spermogonia and the other does not, at least not to any great extent.

The outlook for obtaining any striking results in crossing the two binucleate races is not very promising, inasmuch as no nuclear

[3] In this paper as originally published, Plates IV and V were transposed.

fusions occur in the spores of either race. Two sporidia would develop into a rust like one parent, and the other two would give new generations of the other parent. If it should be found, however, that heterothallism enters into the question, the results of infection work may yet be found interesting, especially when one remembers that one of the uninucleate strains lacks the male structures. The production of a binucleate strain through the inauguration of cell fusions again, by introducing two uninucleate strains into the same plant, is not impossible.

When it comes to crossing the long-cycled forms with each of the short-cycled races there are more grounds for expecting real hybridization. Nuclear fusions occur in the teliospore of the long-cycled orange rust, but whether long-cycledness would persist in a hybrid from a cross with a short-cycled form is, of course, still problematical. The differences in spore sizes, color, and form, the relative abundance of spermogonia, the ability to infect the black raspberry (*Rubus occidentalis*), are some of the features or characters which would mark the results of crossing the different races of the Rubus orange rust, *Gymnoconia interstitialis*.

SUMMARY

With respect to the number of nuclei originally contained in the spores as they are cut off, there are three types to be found in different strains of the short-cycle, or *Caeoma nitens*, forms of the Rubus orange rust. Spores of the first type arise after a fusion between two adjacent cells in the sorus primordium; such spores are binucleate from their origin. Spores of the second type arise from uninucleate cells of the primordium, and without fusions. Such spores are thus uninucleate at their origin. The process by which certain spores may become multinucleate and concomitantly giant spores, constituting the third type of spore, the germination of which has been described, may be discussed in a later paper.

It has been shown that in case of spores of the first type—the binucleate spores—there are no nuclear fusions in the spore, which is contrary to what has been claimed heretofore, and, furthermore, there are no nuclear fusions at any time during germination. As a spore begins to form a germ tube, the two nuclei elongate, at first becoming pear-shaped, with the narrow part pointing toward the germ pore. Further elongation and distortion of the nuclei take place as they migrate side by side, or one slightly behind the other, out into the germ tube. Upon shortening and rounding up again, the nuclei come to lie in the middle of the tube, one beyond the other, and not side by side. During the entire process of germination a nucleole is present in each nucleus and is distinctly visible in well-stained preparations. Division of the two nuclei then occurs, usually simultaneously. The spindles may lie more or less parallel, but the division is not in the true sense conjugate. One nucleus may divide somewhat in advance of the other. After the chromatin masses have separated and come to the poles the pairs of reforming sister nuclei move apart or slide by one another and a median septum is laid down. Each cell of the promycelium at this stage contains two sister nuclei. Soon after these nuclei have rounded up, two septa are laid down, so that the mature promycelium consists of four

uninucleate cells. In the teliospore where a nuclear fusion occurs the promycelium has a two-celled stage with only one nucleus in each cell.

The nucleus of the uninucleate type of spores, after having migrated into the germ tube, divides only once, and the mature promycelium consists of two uninucleate cells. Only two sporidia are formed.

In case of the multinucleate spores, there are likewise no nuclear fusions. After the nuclei have passed out into the very large germ tube they divide, ordinarily about the same time. Their spindles lie at various angles in the tube, tending to lie in the general direction of the tube, depending on whether or not the tube is branched. The degeneration of nuclei in such promycelia frequently occurs, and irregular distribution of the daughter nuclei may take place, but, if not, there will be twice as many cells in the mature promycelium as there were nuclei in the mature spore, so that promycelia from trinucleate spores would produce six sporidia.

The formation of a hybrid between a short-cycle strain whose spores are uninucleate and a short-cycle strain with binucleate spores would not be expected to lead to the development of a new form of the rust.

LITERATURE CITED

(1) Barclay, A.
1891. on the life-history of a remarkable uredine on jasminum grandiflorum, l. (uromyces cunninghamianus, nov. sp.). Linn. Soc. London Trans., Bot. (2) 3: 141–151, illus.

(2) Bary, A. de
1866. morphologie und physiologie der pilze, flechten und myxomyceten. *In* Hofmeister, W., Handbuch der physiologischen Botanik, Bd. 2, Abt. 1, illus. Leipzig.

(3) Blackman, V. H.
1904. on the fertilization, alternation of generations, and general cytology of the uredineae. Ann. Bot. [London] 18: 323–373, illus.

(4) Dodge, B. O.
1918. studies in the genus gymnosporangium—i. notes on the distribution of the mycelium, buffer cells, and the germination of the aecidiospore. Brooklyn Bot. Gard. Mem. 1: 128–140, illus.

(5) ———
1924. uninucleated aecidiospores in caeoma nitens, and associated phenomena. Jour. Agr. Research 28: 1045–1058, illus.

(6) Fromme, F. D.
1914. the morphology and cytology of the aecidium cup. Bot. Gaz. 58: 1–35, illus.

(7) Grove, W. B.
1913. the british rust fungi (uredinales). 412 p., illus. Cambridge.

(8) Harper, R. A.
1900. sexual reproduction in pyronema confluens and the morphology of the ascocarp. Ann. Bot. [London] 14: 321–400, illus.

(9) Hoffmann, A. W. H.
1911. zur entwicklungsgeschichte von endophyllum sempervivi. Centbl. Bakt. [etc.] (II) 32: 137–158, illus.

(10) Kunkel, L. O.
1913. the production of a promycelium by the aecidiospores of caeoma nitens burrill. Bul. Torrey Bot. Club 40: 361–366, illus.

(11) ———
1914. nuclear behavior in the promycelia of caeoma nitens burrili and puccinia peckiana howe. Amer. Jour. Bot. 1: 37–46, illus.

(12) KURSANOV, L. I.
1922. RECHERCHES MORPHOLOGIQUES ET CYTOLOGIQUES SUR LES URÉDINÉES. Bul. Soc. Nat. Moscou (n. s. 1917) 31 (Sect. Biol.): 1–129, illus.
(13) LINDFORS, T.
1924. STUDIEN ÜBER DEN ENTWICKLUNGSVERLAUF BEI EINIGEN ROSTPILZEN AUS ZYTOLOGISCHEN UND ANATOMISCHEN GESICHTSPUNKTEN. Svensk. Bot. Tidskr. 18: 1–84, illus.
(14) MAIRE, R.
1900. L'ÉVOLUTION NUCLÉAIRE CHEZ LES ENDOPHYLLUM. Jour. Bot. 14: 80–97, 369–382, illus.
(15) ———
1902. RECHERCHES CYTOLOGIQUES ET TAXONOMIQUES SUR LES BASIDIOMYCÈTES. Bul. Trimest. Soc. Mycol. France 18 (sup.): 1–209, illus.
(16) MOREAU, F., and MOREAU, MME F.
1918. L'ÉCIDIOSPORE DE L'ENDOPHYLLUM EUPHORBIAE-SILVATICAE (D. C.) WINTER EST-ELLE LE SIÈGE D'UNE KARYOGAMIE? Bul. Trimest. Soc. Mycol. France 33: 97–99, illus.
(17) ——— and MOREAU, MME F.
1918. L'ÉVOLUTION NUCLÉAIRE CHEZ L'ENDOPHYLLUM SEMPERVIVI LÉV. Bul. Trimest. Soc. Mycol. France 33: 70–72, illus.
(18) ——— and MOREAU, MME F.
1919. LES URÉDINÉES DU GROUPE ENDOPHYLLUM. Bul. Soc. Bot. France (sér. 4, t. 19) 66: 14–44, illus.
(19) ———
1919. UNE ANOMALIE DANS L'HISTOIRE NUCLÉAIRE DES SPORES DE L'ENDOPHYLLUM SEMPERVIVI LÉV. Bul. Trimest. Soc. Mycol. France 35: 98–101, illus.
(20) MOREAU, MME F.
1911. SUR L'EXISTENCE D'UNE FORME ÉCIDIENNE UNINUCLÉÉE. Bul. Trimest. Soc. Mycol. France 27: 489–493, illus.
(21) ———
1914. LES PHÉNOMÈNES DE LA SEXUALITÉ CHEZ LES URÉDINÉES. Botaniste 13: 145–284, illus.
(22) ———
1915. NOTE SUR LA VARIÉTÉ UNINUCLÉÉE DE L'ENDOPHYLLUM EUPHORBIAE (D. C.) WINTER. Bul. Trimest. Soc. Mycol. France 31: 68–70, illus.
(23) OLIVE, E. W.
1911. THE NUCLEAR CONDITIONS IN CERTAIN SHORT-CYCLED RUSTS. (Abstract) Science (n. s.) 33: 194.
(24) ——— and WHETZEL, H. H.
1917. ENDOPHYLLUM-LIKE RUSTS OF PORTO RICO. Amer. Jour. Bot. 4: 44–52, illus.
(25) POIRAULT, G.
1913. SUR QUELQUES URÉDINÉES NOUVELLES. Bul. Assoc. Nat. Nice et Alpes-Maritimes 1 (20): 105–108.
(26) ———
1915. SUR QUELQUES CHAMPIGNONS PARASITES RARES OU NOUVEAUX OBSERVÉS DANS LES ALPES-MARITIMES. Bul. Assoc. Nat. Nice et Alpes-Maritimes 2 (3): 7–19.
(27) SAPPIN-TROUFFY, P.
1896. RECHERCHES HISTOLOGIQUES SUR LA FAMILLE DES URÉDINÉES. Botaniste 5: 59–244, illus.
(28) SOPPITT, H. T.
1893. AECIDIUM LEUCOSPERMUM DC. Jour. Bot. [London] 31: 273–274.
(29) WOLF, F. A.
1912. SPORE FORMATION IN PODOSPORA ANSERINA (RABH.) WINTER. Ann. Mycol. 10: 60–64, illus.

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1926